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Lab Resource: Stem Cell Line

Induced pluripotent stem cells derived from a patient with familial idiopathic basal ganglia calcification (IBGC) caused by a mutation in *SLC20A2* gene

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ABSTRACT

Idiopathic basal ganglia calcification (IBGC), also known as Fahr disease or primary familial brain calcifications (PFBC), is a rare neurodegenerative disorder characterized by calcium deposits in basal ganglia and other brain regions, causing neuropsychiatric and motor symptoms. We established human induced pluripotent stem cells (iPSCs) from an IBGC patient. The established IBGC-iPSCs carried *SLC20A2* c.1848G>A mutation (p.W616* of translated protein PiT2), and also showed typical iPSC morphology, pluripotency markers, normal karyotype, and the ability of *in vitro* differentiation into three-germ layers. The iPSC line will be useful for further elucidating the pathomechanism and/or drug development for IBGC.

Resource Table

Unique stem cell line identifier	CIRAi003-A
Alternative name of stem cell line	HPS1036
Institution	Center for iPS Cell Research and Application (CiRA), Kyoto University
Contact information of distributor	Haruhisa Inoue haruhisa@cira.kyoto-u.ac.jp
Type of cell line	iPSC
Origin	Human
Additional origin info	28 year-old, male
Cell source	Peripheral blood mononuclear cells (PBMCs)
Method of reprogramming	Episomal vectors (SOX2, KLF4, OCT4, L-MYC, LIN28 and p53 carboxy-terminal dominant-negative fragment)
Associated disease	Idiopathic basal ganglia calcification (IBGC)
Gene/locus	*SLC20A2*
Method of modification	Not available
Gene correction	NO
Name of transgene or resistance	Not available
Inducible/constitutive system	Not available
Date archived/stock date	Jan, 2015
Cell line repository/bank	RIKEN BioResource Center, Japan http://en.brc.riken.jp/index.shtml
Ethical approval	Ethics Committee of the Department of Medicine and Graduate School of Medicine, Kyoto University (approved No. R0091 and G259), and Gifu university (approved No. 25-65)

Resource utility

IBGC is a rare disease, characterized by massive calcification around brain vessels and various neurological symptoms, the mechanism of which is unknown. IBGC-iPSC will provide endothelial cells or neural cells and help the elucidation of the pathomechanism in the neurovascular system, one of key targets to treat neurological disorders.

Resource details

Idiopathic basal ganglia calcification (IBGC) is a rare genetic condition characterized by symmetric calcification in the basal ganglia and other brain regions. The clinical symptoms of IBGC include neuropsychiatric

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Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Similar to human embryonic stem cells	Fig. 1 panel A
Phenotype	Immunocytochemisty	Assess staining of pluripotency markers: NANOG and SSEA-4	Fig. 1 panel A
	Flow cytometry	SSEA-4 87.6%	Fig. 1 panel E
Genotype	Karyotype (G-banding) and resolution	46XY, Resolution 450–500	Fig. 1 panel C
Identity	Microsatellite PCR (mPCR)	Not performed	Not performed
	STR analysis	16 loci, matched	Supplementary Table 1
Mutation analysis (IF APPLICABLE)	Sequencing	Heterozygous, *SLC20A2* mutation (c.1848G>A, p.W616* of translated protein PiT2)	Fig. 1 panel D
	Southern Blot OR WGS	Not performed	Not performed
Microbiology and virology	Mycoplasma	Mycoplasma testing by luminescence method (MycoAlert™ Mycoplasma Detection Kit). Negative	Not shown but available with author
Differentiation potential	Embryoid body formation OR Teratoma formation OR Scorecard	Describe expression of genes in embryod bodies: SOX-17, αSMA, and βIII-tubulin	Fig. 1 panel B
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	HIV1 (negative), HIV2 (not performed), Hepatitis B (negative), and Hepatitis C (negative)	Not shown but available with author
Genotype Additional info (OPTIONAL)	Blood group genotyping	Not available	Not available
	HLA tissue typing	Not available	Not available

symptoms, dementia, psychosis, seizures, or chronic headache, and normal serum levels of calcium, phosphate, alkaline phosphatase and parathyroid hormone. Typical age at clinical onset is between 20 and 50 years, and most individuals are asymptomatic (Nicolas et al., 2015). The neuropathological hallmark of IBGC is calcification of peri- and intra-vascular space of capillaries in the basal ganglia, dentate nuclei of the cerebellum and white matter. IBGC is frequently inherited in an autosomal dominant manner. Approximately half of the cases of familial IBGC associate with mutations in *SLC20A2*, which encodes the type III Na^+-dependent inorganic phosphate transporter PiT2 (Yamada et al., 2014). The mutations are predicted to result in a loss of function of inorganic phosphate transport. *SLC20A2* knockout mice show cerebrovascular calcification. We generated induced pluripotent stem cells (iPSCs) from an IBGC patient with *SLC20A2* mutation (c.1848G>A, p.W616* of translated protein PiT2) Table 1. Established IBGC-iPSCs showed positive staining of pluripotency markers (Fig. 1A). Three germ layer differentiation capacity was showed in the *in vitro* differentiation followed by immunohistochemistry staining of the mesodermal marker (alpha smooth muscle actin: αSMA), the endodermal marker (SOX17), and the ectodermal marker (βIII-tubulin) (Fig. 1B), Table 2. Established IBGC-iPSCs also maintained a normal karyotype after reprogramming process (Fig. 1C), and retained *SLC20A2* mutation (c.1848G>A, p.W616* of translated protein PiT2) (Fig. 1D. By flow cytometry, 66.1% of iPSCs were positive for SSEA-4 (Fig. 1E).

Materials and methods

Ethics statements

Generation and use of human iPSCs was approved by the Ethics Committee of the Department of Medicine and Graduate School of Medicine, Kyoto University and Gifu University, and all methods were performed in accordance with the approved guidelines. Formal informed consent was obtained from the patient.

Establishment of iPSCs

Peripheral blood mononuclear cells (PBMCs) from an IBGC patient were cultured in StemFit/AK03 A, B without C (Ajinomoto, Tokyo, Japan) supplemented with IL-6, SCF, TPO, Flt-3L, IL-3, G-CSF (Wako Pure Chemical Industries, Osaka, Japan). After 7 days of culture, reprogramming factors, including SOX2, KLF4, OCT4, L-MYC, LIN28 and p53 carboxy-terminal dominant-negative fragment, were transduced into PBMCs with episomal vectors (Okita et al., 2013). PBMCs were cultivated in disseminated onto MEF feeder-cell with Primate ES cell medium (ReproCELL Inc., Yokohama, Japan). iPSCs colonies were picked up, and the iPSC maintenance method was changed to a feeder-free system by using StemFit medium (AK02N, Ajinomoto, Tokyo, Japan) on iMatrix-511 (Nippi, Tokyo, Japan)-coated plates.

Genotyping by sequencing analysis

Genomic DNA was extracted from PBMCs and iPSCs with Purelink Genomic DNA Kits (Invitrogen, Thermo Fisher Scientific, Waltham, MA). The SLC20A2 coding region was amplified by using KOD-plus-Neo (Toyobo, Osaka, Japan) and Veriti Thermal Cycle (Thermo Fisher Scientific), followed by direct sanger sequence (3700 Genetic Analyzer; formerly Applied Biosystems, Thermo Fisher Scientific).

In vitro differentiation

The established iPSCs were harvested by TrypLE express (Thermo Fisher Scientific) and cultivated in DMEM/F12/Glutamax (Thermo Fisher Scientific), 20% knockout serum replacement (KSR; Thermo Fisher Scientific), 10 μM Y-27632(Nacalai tesque, Kyoto, Japan) on 0.5% Lipidure (Nichiyu, Tokyo, Japan)-coated U-shape-bottom 96-well-plates (Greiner bio-one, Kremsmünster, Austria) for generation of aggregates. On day 7, aggregates were seeded onto matrigel-coated 96-well-plates and differentiated into three-germ layer in DMEM plus 10% fetal bovine serum (Thermo Fisher Scientific).

Immunocytochemistry

Cells were fixed with 4% paraformaldehyde and blocked with PBST containing 5% Blocking One Histo (Nacalai tesque, Kyoto, Japan). DAPI (Thermo Fisher Scientific) was used for nuclei. Fluorescence imaging was performed by fluorescence microscope BIOREVO BZ-9000 (Keyence, Osaka, Japan).

Karyotype analysis

Karyotyping was performed by LSI Medience Corporation (Tokyo, Japan).

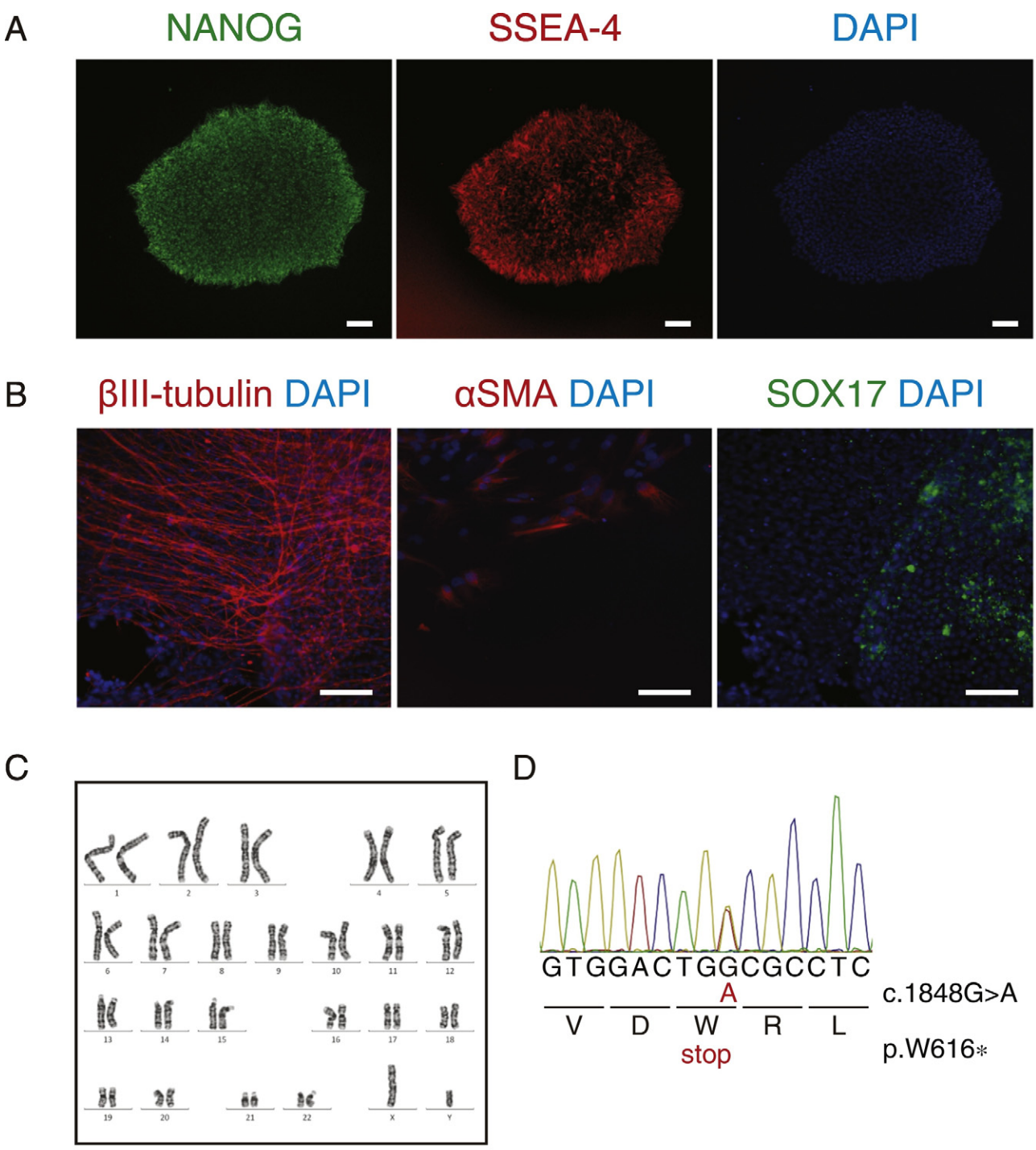

Table 2
Summary of antibodies and primers.

Antibodies used for immunocytochemistry/flow cytometry			
	Antibody	Dilution	Company Cat# and RRID
Pluripotency markers	Rabbit anti-NANOG	1:500	Cosmo Bio Co Cat# RCAB0003P, RRID: AB_1962353
Pluripotency markers	Mouse anti-SSEA-4	1:1000	Millipore Cat# MAB4304, RRID: AB_177629
Ectoderm differentiation markers	Mouse anti-βIII-tubulin	1:500	Millipore Cat# CBL412X, RRID: AB_1977541
Mesoderm differentiation markers	Mouse anti-αSMA	1:100	DAKO Cat# M0851, RRID: AB_2223500
Endoderm differentiation markers	Goat anti-SOX17	1:300	R and D Systems Cat# AF1924, RRID: AB_355060
Secondary antibodies	Donkey anti-Goat IgG Alexa Fluor 488	1:1000	Thermo Fisher Scientific Cat# A-11055, RRID: AB_142672
Secondary antibodies	Goat anti-Rabbit IgG Alexa Fluor 488	1:1000	Thermo Fisher Scientific Cat# A-11034, RRID: AB_2576217
Secondary antibodies	Goat anti-Mouse IgG Alexa Fluor 546	1:1000	Thermo Fisher Scientific Cat# A-11030, RRID: AB_2534089
Primers			
	Target	Forward/Reverse primer (5′-3′)	
Genotyping	*SLC20A2*	*Fw:GCTGAAGAGAAGAATCCCCAAAC* *Rv:GGTGAACAGTGTGGGATGGAG*	

Flow cytometry analysis

iPSCs were dissociated into single cell by using Accumax (Innovative Cell Technologies, San Diego, CA), and were incubated at 1.0×10^6 cells/-ml in PBS with 2% FBS and 20 μl SSEA-4 APC conjugated monoclonal antibody (BD Biosciences, Franklin Lakes, NJ) for 30 min at 4 °C. After staining, the cells were washed twice in PBS with 2% FBS and were analyzed on a FACS Aria II (BD Biosciences) high-speed cell sorter using the 647 nm excitation and 100 μm nozzle. Unstained controls were also analyzed as negative control to exclude data from non-specific fluorescence.

DNA fingerprinting

STR analysis was performed by using AuthentiFiler PCR Amplification Kit (Thermo Fisher Scientific).

Mycoplasma test

iPSCs were confirmed to be mycoplasma-negative using the MycoAlert kit (Lonza, Basel, Switzerland) in accordance with the manufacturer's instructions.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scr.2017.07.028.

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References

Nicolas, G., Charbonnier, C., de Lemos, R.R., Richard, A.C., Guillin, O., Wallon, D., Legati, A., Geschwind, D., Coppola, G., Frebourg, T., Campion, D., de Oliveira, J.R.M., Hannequin, D., Anheim, M., Ayrignac, X., Azulay, J.P., Derache, N., Durif, F., Guyant-Maréchal, L., Hodille-Renaud, M., Labauge, P., Le Ber, I., Lefaucheur, R., Maltête, D., Pariente, J., Rouault, O., Thauvin-Robinet, C., Rudolf, P., Tranchant, C., Verny, C., 2015. Brain calcification process and phenotypes according to age and sex: lessons from SLC20A2, PDGFB, and PDGFRB mutation carriers. Am. J. Med. Genet. Part B Neuropsychiatr. Genet. 168, 586–594.

Okita, K., Yamakawa, T., Matsumura, Y., Sato, Y., Amano, N., Watanabe, A., Goshima, N., Yamanaka, S., 2013. An efficient nonviral method to generate integration-free human-induced pluripotent stem cells from cord blood and peripheral blood cells. Stem Cells 31, 458–466.

Yamada, M., Tanaka, M., Takagi, M., Kobayashi, S., Taguchi, Y., Takashima, S., Tanaka, K., Touge, T., Hatsuta, H., Murayama, S., Hayashi, Y., Kaneko, M., Ishiura, H., Mitsui, J., Atsuta, N., Sobue, G., Shimozawa, N., Inuzuka, T., Tsuji, S., Hozumi, I., 2014. Evaluation of SLC20A2 mutations that cause idiopathic basal ganglia calcification in Japan. Neurology 82, 705–712.

Fig. 1. Characterization of the iPSC line. (A) Established IBGC-iPSCs displayed a typical round-shaped colony. Immunocytochemical staining of iPSCs showed positive stainning of pluripotency markers NANOG (green) and SSEA-4 (red). Scale bars: 200 μm. (B) Spontaneously differentiated cells after embryoid body formation showed positive staining for each marker of three-germ layer: βIII-tubulin (red), alpha smooth muscle actin: αSMA (red), and SOX17 (green). Scale bars: 100 μm. (C) Karyotype analysis of patient iPSCs showed a normal karyotype of 46 XY. (D) Sanger sequence of the *SLC20A2* gene in iPSCs showed a heterozygous c.1848G > A (p.W616*) mutation. (E) FACS analysis with evaluation of SSEA-4 staining.